Infections in de novo kidney transplant recipients treated with the RANKL inhibitor denosumab

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Abstract: BACKGROUND: Infections are a major cause of morbidity and mortality in kidney allograft recipients. In this posthoc analysis of a randomized clinical trial which tested the effect of denosumab on bone mineral density we assessed the impact of this drug on the incidence and severity of infections in the first year after kidney transplantation. METHODS: In this clinical trial we randomized 90 de novo kidney transplant recipients shortly after transplantation to either denosumab on top of standard treatment (calcium and vitamin D) (n=46), or to standard treatment alone (n=44). Among all adverse events we analyzed all infections that occurred within the first year after transplantation, and compared their incidence and severity in both groups. RESULTS: Overall we identified more infections (n=146) in the denosumab group than in the control group (n=99). The most common infections were urinary tract infection (cystitis) (34.9% vs 25.2%), CMV viremia (17.8% vs 24.2%), flu-like syndrome (11.6% vs 14.1%), polyoma (BK) viremia (8.2% vs 11.1%), and herpes simplex infections (5.5% vs 4.0%). Episodes of urinary tract infection (cystitis) occurred more often in the denosumab than in the control group (51 vs 25 episodes in 24 vs 11 patients, p=0.008), whereas episodes of transplant pyelonephritis or urosepsis were not more frequent (3 vs 5 episodes). CONCLUSIONS: This post-hoc analysis reveals that treatment with denosumab to prevent bone loss in first-year kidney transplant recipients was associated with more frequent episodes of urinary tract infections, whereas other infections occurred with similar frequency in both treatment groups.

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Infections in de novo Kidney Transplant Recipients Treated with the RANKL Inhibitor Denosumab

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Disclosures

RPW reports personal fees from Amgen, outside the submitted work. NG reports personal fees from Boehringer Ingelheim GmbH, medac GmbH, and Bayer, outside of the submitted work.

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Abstract

Background: Infections are a major cause of morbidity and mortality in kidney allograft recipients. In this posthoc analysis of a randomized clinical trial which tested the effect of denosumab on bone mineral density we assessed the impact of this drug on the incidence and severity of infections in the first year after kidney transplantation.

Methods: In this clinical trial we randomized 90 de novo kidney transplant recipients shortly after transplantation to either denosumab on top of standard treatment (calcium and vitamin D) (n=46), or to standard treatment alone (n=44). Among all adverse events we analyzed all infections that occurred within the first year after transplantation, and compared their incidence and severity in both groups.

Results: Overall we identified more infections (n=146) in the denosumab group than in the control group (n=99). The most common infections were urinary tract infection (cystitis) (34.9% vs 25.2%), CMV viremia (17.8% vs 24.2%), flu-like syndrome (11.6% vs 14.1%), polyoma (BK) viremia (8.2% vs 11.1%), and herpes simplex infections (5.5% vs 4.0%). Episodes of urinary tract infection (cystitis) occurred more often in the denosumab than in the control group (51 vs 25 episodes in 24 vs 11 patients, p=0.008), whereas episodes of transplant pyelonephritis or urosepsis were not more frequent (3 vs 5 episodes).

Conclusions: This *post-hoc* analysis reveals that treatment with denosumab to prevent bone loss in first-year kidney transplant recipients was associated with more frequent episodes of urinary tract infections, whereas other infections occurred with similar frequency in both treatment groups.

Introduction

A major cause of morbidity and mortality in kidney transplant recipients are infections [1]. The risk of infection is influenced by a variety of factors including immunosuppressive therapy, treatment of rejection episodes, reactivation of a previous latent infection, presence of indwelling catheters, nutritional status and uncontrolled glycemia in patients with diabetes mellitus [2-4]. The impact of infections can be reduced by careful pre-transplant screening of recipients and donors, vaccinations, post-transplant infection prophylaxis and appropriate dosing of the immunosuppressive treatment [2,5].

The incidence and the type of infections that occur after transplantation typically follow a characteristic pattern with regard to time after transplantation [6]. However, the introduction of novel therapeutic agents, particularly drugs which affect the immune system or the host defense, may lead to unexpected or novel infectious complications. Examples are the increasing incidence of BK virus nephropathy [7,8] that can be attributed at least in part to the introduction of immunosuppressive drugs such as tacrolimus and mycophenolate, or the higher risk for Epstein-Barr virus-associated lymphomas in patients treated with the novel immunosuppressant belatacept [9,10].

Kidney transplant recipients often have osteopenia or osteoporosis which leads to an increased risk for fractures [11]. Therapeutic options to improve the loss of bone mass include supplementation with calcium and vitamin D and its analogues [12,13], and treatment with bisphosphonates [14-17]. In a randomized clinical trial we have recently shown that denosumab - a fully human monoclonal antibody against Receptor Activator of Nuclear Factor □B Ligand (RANKL) – effectively increased bone mineral density when given during the first year after kidney transplantation [18]. In that trial we noticed that denosumab unexpectedly increased the incidence of urinary tract infections. We therefore sought to analyze in detail all infectious

episodes that occurred during this clinical trial, and to obtain more information regarding the type and course of the infectious episodes.

Materials and Methods

Study design

The trial design has previously been described in detail (NCT01377467) [18]. In brief, this academic study was a 1-year prospective single-center, randomized, parallel-group, open-label clinical trial in 90 de novo kidney transplant recipients. Patients were randomized to receive subcutaneous injections of 60 mg denosumab at baseline and after 6 months (n=46) or no treatment (n=44). All patients received calcium (1000 mg) and vitamin D (800 IU or more) supplementation as a standard treatment. All patients were followed up in the same institution in the first year after transplantation. Study visits were performed at baseline and months 0.5, 1, 2, 3, 6, and 12. All clinical and routine laboratory values and all adverse events including infections were captured with a secured web-based case report form (secuTrial®, interActive Systems, Berlin, Germany).

All infectious episodes were carefully reviewed to document their type, severity and duration. In particular, all microbiology, virology and serology results were reviewed in all patients to classify the infections.

Urinary tract infection (cystitis) was defined as an episode of local urinary symptoms such as dysuria, frequency or urgency in combination with the presence of $>10^5$ CFU/mL on urine culture with or without leukocyturia. Episodes of urinary tract infection with negative urine culture were also counted if clinically suggestive and empirically treated with antibiotics. The severity of urinary tract infection was defined according to the Common Terminology Criteria for Adverse Events (CTCAE) Version 4.0.

Transplant procedure and posttransplant management

Among all 90 kidney transplants 44 were from deceased donors (48.9%) and 46 from living donors (51.1%). Preemptive transplantation was performed in 17 patients (18.9%) and repeat transplantation in 14 patients (15.6%). During transplantation a pigtail catheter was inserted routinely to stent the transplant ureter, and a Foley catheter to drain the bladder. The Foley catheter was removed 4 to 6 days after surgery and the pigtail catheter 6 weeks posttransplantation.

Patients received induction treatment with basiliximab (68.9%) or anti-thymocyte globulin (30.0%), together with triple immunosuppression therapy which included a calcineurin inhibitor (70% tacrolimus, 30% cyclosporine), mycophenolate and corticosteroids. Corticosteroids were tapered and successfully removed from 3 patients (3.3%) after 6 months, and from another 42 patients (46.7%) after 12 months.

All patients received a daily chemoprophylaxis with trimethoprim (80 mg) and sulfamethoxazole (400 mg) for *Pneumocystis jirovecii* and urinary tract infections in the first 6 months posttransplant. CMV was approached with a preemptive strategy. Thus, CMV prophylaxis with daily valganciclovir (900 mg, adapted for renal function) for the first 3 months was only prescribed to high-risk (D+/R-) and intermediate risk patients (D+/R+; D-/R+) if they received anti-thymocyte globulin induction treatment.

Statistical analysis

Categorical data were analyzed with the chi-square test or Fisher's exact test to check for independence of 2 variables. For the analysis of stratified categorical data, the Mantel-Haenszel test was used. The exact Wilcoxon rank sum test was used to compare for differences in ordinal or interval scaled, not normally distributed variables.

Results

The study population included 90 kidney transplant recipients. Patients were randomized after a mean (SD) of 15.7 (6.4) days after transplantation to denosumab treatment (n=46) at a dose of 60 mg subcutaneously at baseline and at 6 months or no treatment (n=44). Two patients did not receive denosumab and another 2 patients received only the baseline injection of denosumab.

The baseline patient characteristics are shown in Table 1. The 2 study groups were generally well balanced, except that the denosumab group had more men and more living donor transplants. Both study groups were treated with similar immunosuppression. The CMV risk profile was similar in both groups.

During the 12-month study period we recorded a total of 349 adverse events (AE) in the denosumab group (7.6 AE per patient) and 273 AE in the control group (6.2 AE per patient), of which 146 (41.8%) and 99 (36.3%), respectively, were infections. The number of infections per patient tended to be higher in the denosumab than in the control group (median 3 vs 2 infections, p=0.068), but the number of patients with any infection was not different in the 2 treatment groups (41 vs 37; p=0.482).

Table 2 shows that the most common infections were urinary tract infections (cystitis) (31.0%), CMV viremia (20.4%), flu-like syndrome (12.6%), polyoma (BK) viremia (9.3%), and herpes simplex infections (4.9%). Of importance, episodes of urinary tract infection occurred in more patients in the denosumab than in the control group (51 vs 25 episodes), whereas the number of episodes with CMV viremia (26 vs 24), flu-like syndrome (17 vs 14), polyoma (BK) viremia (12 vs 11) and herpes simplex infection (8 vs 4) did not differ.

We noticed 5 acute rejection episodes in 5 patients in the denosumab group and 4 acute rejection episodes in 3 patients in the control group. The cumulative amount of corticosteroids

over the 12-month study period was similar, amounting to 3.760 ± 1.445 g in the denosumab and 3.974 ± 1.727 g in the control group.

Urinary tract infections (cystitis) and pyelonephritis/urosepsis

Episodes of urinary tract infection (cystitis) occurred in more patients in the denosumab than in the control group (24 vs 11 patients, odds ratio (OR) 3.3 [95% CI 1.3-8.0], p=0.008), whereas transplant pyelonephritis and urosepsis showed no difference in the 2 groups (3 vs 5 patients, p=0.480). Clinically, the 51 episodes of urinary tract infection (cystitis) in the denosumab group were of mild (3.9%), moderate (76.5%), or severe (19.6%) degree. The 25 episodes in the control group were of moderate (56.0%) or severe (44.0%) degree. Thus, the fraction of severe episodes of urinary tract infections were higher in the control than in the denosumab group (p=0.025). Figure 1 displays the cumulative incidence of the urinary tract infections in both groups. In the denosumab group there were 4/51 urinary tract infections and in the control group 0/25 infection that occurred early in the first 4 to 6 days after transplantation while the Foley catheter was in place. In the denosumab group 20/51 urinary tract infections and 6/25 infections in the control group occurred in the first 6 weeks after transplantation while the ureteral stent was in place. In the denosumab group, 18/51 urinary tract infections and 7/25 infections in the control group occurred between 6 and 12 months after transplantation, ie at the time when antibiotic prophylaxis had been stopped. Overall, two-thirds of the urinary tract infections occurred in the first 6 months after transplantation, and did not coincide with the removal of the pigtail catheter 6 weeks posttransplant, or follow the application of denosumab at baseline and after 6 months, or follow the removal of the antibiotic prophylaxis. In addition there was no difference in the duration of the urinary drainage with the Foley catheter or the ureteral stents in both groups.

Women tended to be affected by urinary tract infections more often than men (16/33 vs 19/57) (p=0.155). As gender was not equally distributed between the treatment groups, the OR of 3.3 for urinary tract infections under denosumab treatment raised to 4.8 (95% CI 1.7-13.5) when controlling for gender. Other parameters did not seem to have an influence on the frequency of urinary tract infections. Thus, neither the type of donation (living vs deceased; p=0.361) nor the type of induction therapy (ATG vs basiliximab; p=0.424) were associated with the occurrence of urinary tract infections.

The urine cultures were positive in all 25 episodes of urinary tract infection in the control group (100%), whereas only 42 of 51 cultures were positive in the denosumab group (82%), despite a clear clinical diagnosis of urinary tract infection. The spectrum of urine bacteria was similar in the denosumab and control group (Table 3). The most frequent bacteria included *Escherichia coli* (n=23), *Klebsiella pneumonia* (n=12) and *Enterococcus faecalis* (n=9). *Escherichia coli* (17/51 vs 6/25 urinary tract infections) and *Enterococcus faecalis* (8/51 vs 1/25 urinary tract infections) occurred more frequently in the denosumab than in the control group. The pathogen for all 8 episodes of pyelonephritis was *Escherichia coli*.

Urinary tract infections were treated with oral (n=55) or intravenous antibiotics (n=21). Oral antibiotics were prescribed for 41 (80.4%) infections in the denosumab group and for 14 infections in the control group (56.0%). In the denosumab group 10 (19.6%) infections were treated with intravenous therapy vs 11 (44.0%) infections in the control group.

CMV viremia

The details regarding CMV viremia are shown in Table 4. CMV viremia occurred with similar frequency in both groups (20 vs 18 patients, p=0.805). The median time to CMV viremia occurrence after transplantation was similar in both groups. In each group 5 primary

infections occurred after stopping the CMV prophylaxis with valganciclovir. The distribution of CMV viremia was significantly different for low, intermediate and high risk constellation (p=0.003), with most viremias occurring in the intermediate and high risk constellations. CMV disease occurred in none of the patients in the denosumab group and in 1 patient in the control group. This patient developped diarrhea, and the histologic specimen from the colonoscopy confirmed the diagnosis of CMV colitis. Oral valganciclovir was prescribed to 23 viremias in the denosumab group and 11 viremias in the control group. Intravenous ganciclovir was used in 1 case in the denosumab group and 6 cases in the control group. Two and 7 cases with intermediate risk constellation received no treatment due to a very low virus replication (<2000 copies/ml). In summary, denosumab did not appear to alter the incidence, severity and duration of CMV infection.

Flu-like syndromes

Flu-like syndromes occurred in 12 patients in the denosumab group and 13 patients in the control group (p=0.714). A total of 24 of the infections occurred between October and April and 7 infections between May and September, corresponding to the seasonal variation.

Polyoma (BK) viremia

The details regarding BK viremia are shown in Table 4. The number of patients with BK viremia was not different between the 2 groups, whereas the median time to BK viremia after transplantation occurred significantly earlier in the control group (p=0.049). One case of biopsy-proven BK virus nephropathy occurred in the denosumab group and none in the control group. In the denosumab group, 5 infections occurred in patients with ATG induction and 6 infections with basiliximab induction. In the control group, 4 infections occurred in

patients treated with ATG and 7 infections when treated with basiliximab. In summary, denosumab did not appear to significantly alter the incidence, severity and duration of BK virus infection.

Herpes simplex type 1 and herpes zoster infections

Herpes simplex type 1 (HSV 1) infections occurred with similar frequency in the denosumab and control groups (5 vs 4, p=0.714). One case of a disseminated HSV 1 infection occurred in the denosumab group and 1 case of herpes oesophagitis (HSV 1) occurred in the control group. Both cases were successfully treated with oral valaciclovir for 10 days. The other cases were classical herpes labialis infections.

One patient in the denosumab group and 2 patients in the control group received oral valaciclovir as primary therapy for herpes labialis and 6 patients in the denosumab group and none in the control group received local therapy with topical acyclovir. One patient in the control group received neither valaciclovir nor acyclovir.

There were 3 cases of herpes zoster in the denosumab group and none in the control group, suggesting that denosumab could promote these infections. None of the cases was a disseminated infection. The localization along the dermatomas was in the face, thoracic and on the leg. All 3 cases were treated with oral valaciclovir for 10 days and none of the patient had a relapse.

Other relevant infections

Wound infections (4 vs 0) as well as cutaneous infections (4 vs 0) were seen more often in the denosumab group than in the control group. Thus, although the number of wound and cutaneous infections was small, it appears that denosumab may promote these infections.

Discussion

Denosumab is a new and first-in-class anti-osteoporotic drug which is highly effective in preventing vertebral and non-vertebral fractures in postmenopausal women with osteoporosis [19] and has shown superior efficacy when compared with bisphosphonates in this patient population [20]. Although generally well tolerated, infections involving the gastrointestinal tract, the renal and urinary system and the skin were shown to occur slightly more often in patients treated with denosumab compared with placebo [21-24]. Whether kidney transplant recipients under immunosuppressive treatment are at higher risk for infection when treated with denosumab has not been studied yet [6,25].

The posthoc analysis of our POSTOP study revealed a higher number of infections in denosumab-treated renal transplant recipients compared with patients in the control group. In particular, denosumab treatment led to a doubling of the number of urinary tract infections (cystitis), whereas other types of infections, particularly opportunistic infections with CMV and BK virus were not more common with denosumab treatment. Such a high incidence of urinary tract infections was not seen in postmenopausal women treated with denosumab [19], suggesting that immunosuppressed de novo renal transplant recipients are more vulnerable to this type of infection. Specific and nonspecific immune response may be influenced by the RANKL system.

The mechanisms which lead to such a high number of urinary tract infections are probably multifactorial. It has been suggested that the inhibition of the RANK/RANKL pathway with denosumab could decrease the resistance to microbial organisms by unknown mechanisms [26,27]. Renal transplant patients are known to be particularly susceptible to urinary tract infections due to structural alterations of the urinary tract which are inherent to the transplant procedure, reflux, bladder dysfunction, and the routine insertion of pigtail ureteral and bladder catheters. It is known, that vitamin D can have immunomodulatory activities. In our study, the serum levels of 25-(OH)-vitamin D and 1, 25-$(OH)_2$-vitamin D progressively increased in both groups to similar levels at 12 months, suggesting that the difference in urinary tract infections in the 2 groups cannot be explained with the immunomodulatory activities of vitamin D[18]. Furthermore, in the setting of strong triple immunosuppression in the first months after transplantation denosumab may contribute to a further increase in the incidence of these infections.

Fortunately, the urinary tract infections in both groups represented mostly benign episodes of cystitis which could easily be managed with antibiotics, and transplant pyelonephritis or urosepsis were not more common in the denosumab group. Furthermore, the severity and duration of these infections was not aggravated with denosumab. Somewhat surprising is the fact that 18% of the urine cultures were negative in the densoumab group, whereas there were no negative cultures in the control group. Thus we cannot exclude that some of these clinically diagnosed episodes of urinary tract infection in the denosumab group were in fact abacterial, or caused by less traditional microorganisms. Nevertheless, the number of urinary tract infections was significantly higher in the denosumab group even after exclusion of the cases with negative culture. Thus it is advisable to pay particular attention to the occurrence of urinary tract infections when denosumab is used early after renal transplantation,

particularly since these infections are 1 of the major causes of morbidity and rehospitalization after kidney transplantation.

The incidence of pyelonephritis and urosepsis in the first year after kidney transplantation can be as high as 9-14% and 3-7%, respectively [28-31]. As mentioned, the rate of pyelonephritis and urosepsis was comparable in both study arms, with 3 episodes (6.5%) in the denosumab group and 5 episodes (11.4%) in the control group. The routine use of antibiotic prophylaxis with trimethoprim-sulfamethoxazole in the first 6 months post-transplant may have prevented a higher incidence of more severe urinary tract infections, as it has been shown in other studies [32]. Of note, in patients with urinary tract infection the spectrum of urine bacteria was similar in the denosumab and the control group. E. coli, Klebsiella pneumoniae and Enterococcus faecalis represented the most common infectious agents, as it is seen in the general transplant population [33,34]. Since only 4 patients in the denosumab group and 3 patients in the control group had diabetes, we could not make a firm statement regarding the known association between diabetes mellitus and urinary tract infections.

CMV infection is the most common opportunistic infection in kidney transplant patients, occurring in approximately 8% of recipients [35]. Risk factors associated with CMV infection include the use of T cell depleting antibodies, the presence of rejection, concurrent infections and donor sero-positivity [36]. In our study, the use of denosumab did not increase the incidence of CMV infection in the treatment group. Likewise, denosumab did not appear to increase the incidence and severity of BK viremia and BK nephropathy. Because all other infections occurred only in very low number of patients, no definitive conclusion regarding the influence of denosumab on these infections can be drawn. However, herpes zoster as well as skin and wound infections appeared to occur more commonly in denosumab-treated patients.

In conclusion, posttransplant treatment with denosumab to prevent bone loss is associated with more frequent episodes of urinary tract infection, whereas transplant-specific infections

occurred with similar frequency in both groups. Caution regarding urinary tract infections and possibly wound, skin and herpes zoster infections is advised when using denosumab early after renal transplantation when immunosuppression is at the highest level.

References

1. Fishman JA. Introduction: infection in solid organ transplant recipients. *Am J Transplant.* 2009;9 Suppl 4:S3-6.
2. Karuthu S, Blumberg EA. Common infections in kidney transplant recipients. *Clin J Am Soc Nephrol.* 2012;7(12):2058-2070.
3. Oriol I, Sabe N, Melilli E, et al. Factors influencing mortality in solid organ transplant recipients with bloodstream infection. *Clin Microbiol Infect.* 2015;21(12):1109-1114.
4. Fishman JA, Issa NC. Infection in organ transplantation: risk factors and evolving patterns of infection. *Infect Dis Clin North Am.* 2010;24(2):273-283.
5. KDIGO clinical practice guideline for the care of kidney transplant recipients. *Am J Transplant.* 2009;9 Suppl 3:S1-155.
6. Fishman JA. Infection in solid-organ transplant recipients. *N Engl J Med.* 2007;357(25):2601-2614.
7. Gonzalez S, Escobar-Serna DP, Suarez O, Benavides X, Escobar-Serna JF, Lozano E. BK Virus Nephropathy in Kidney Transplantation: An Approach Proposal and Update on Risk Factors, Diagnosis, and Treatment. *Transplant Proc.* 2015;47(6):1777-1785.
8. Jamboti JS. BK virus nephropathy in renal transplant recipients. *Nephrology (Carlton).* 2016;21(8):647-54.
9. Vincenti F, Charpentier B, Vanrenterghem Y, et al. A phase III study of belatacept-based immunosuppression regimens versus cyclosporine in renal transplant recipients (BENEFIT study). *Am J Transplant.* 2010;10(3):535-546.
10. Wekerle T, Grinyo JM. Belatacept: from rational design to clinical application. *Transpl Int.* 2012;25(2):139-150.

11. Vautour LM, Melton LJ, 3rd, Clarke BL, Achenbach SJ, Oberg AL, McCarthy JT. Long-term fracture risk following renal transplantation: a population-based study. *Osteoporos Int.* 2004;15(2):160-167.

12. Josephson MA, Schumm LP, Chiu MY, Marshall C, Thistlethwaite JR, Sprague SM. Calcium and calcitriol prophylaxis attenuates posttransplant bone loss. *Transplantation.* 2004;78(8):1233-1236.

13. De Sevaux RG, Hoitsma AJ, Corstens FH, Wetzels JF. Treatment with vitamin D and calcium reduces bone loss after renal transplantation: a randomized study. *J Am Soc Nephrol.* 2002;13(6):1608-1614.

14. Grotz W, Nagel C, Poeschel D, et al. Effect of ibandronate on bone loss and renal function after kidney transplantation. *J Am Soc Nephrol.* 2001;12(7):1530-1537.

15. Jeffery JR, Leslie WD, Karpinski ME, Nickerson PW, Rush DN. Prevalence and treatment of decreased bone density in renal transplant recipients: a randomized prospective trial of calcitriol versus alendronate. *Transplantation.* 2003;76(10):1498-1502.

16. Torregrosa JV, Fuster D, Gentil MA, et al. Open-label trial: effect of weekly risedronate immediately after transplantation in kidney recipients. *Transplantation.* 2010;89(12):1476-1481.

17. Smerud KT, Dolgos S, Olsen IC, et al. A 1-year randomized, double-blind, placebo-controlled study of intravenous ibandronate on bone loss following renal transplantation. *Am J Transplant.* 2012;12(12):3316-3325.

18. Bonani M, Frey D, Brockmann J, et al. Effect of Twice-Yearly Denosumab on Prevention of Bone Mineral Density Loss in De Novo Kidney Transplant Recipients: A Randomized Controlled Trial. *Am J Transplant.* 2016;16(6):1882-1891.

19. Cummings SR, San Martin J, McClung MR, et al. Denosumab for prevention of fractures in postmenopausal women with osteoporosis. *N Engl J Med.* 2009;361(8):756-765.

20. Brown JP, Prince RL, Deal C, et al. Comparison of the effect of denosumab and alendronate on BMD and biochemical markers of bone turnover in postmenopausal women with low bone mass: a randomized, blinded, phase 3 trial. *J Bone Miner Res.* 2009;24(1):153-161.

21. Watts NB, Roux C, Modlin JF, et al. Infections in postmenopausal women with osteoporosis treated with denosumab or placebo: coincidence or causal association? *Osteoporos Int.* 2012;23(1):327-337.

22. Smith MR, Egerdie B, Hernandez Toriz N, et al. Denosumab in men receiving androgen-deprivation therapy for prostate cancer. *N Engl J Med.* 2009;361(8):745-755.

23. Mok CC, Ho LY, Ma KM. Switching of oral bisphosphonates to denosumab in chronic glucocorticoid users: a 12-month randomized controlled trial. *Bone.* 2015;75:222-228.

24. Curtis JR, Xie F, Yun H, Saag KG, Chen L, Delzell E. Risk of hospitalized infection among rheumatoid arthritis patients concurrently treated with a biologic agent and denosumab. *Arthritis Rheumatol.* 2015;67(6):1456-1464.

25. Halloran PF. Immunosuppressive drugs for kidney transplantation. *N Engl J Med.* 2004;351(26):2715-2729.

26. Ferrari-Lacraz S, Ferrari S. Do RANKL inhibitors (denosumab) affect inflammation and immunity? *Osteoporos Int.* 2011;22(2):435-446.

27. Pacifici R. Osteoimmunology and its implications for transplantation. *Am J Transplant.* 2013;13(9):2245-2254.

28. Lyerova L, Viklicky O, Nemcova D, Teplan V. The incidence of infectious diseases after renal transplantation: a single-centre experience. *Int J Antimicrob Agents.* 2008;31 Suppl 1:S58-62.

29. Maraha B, Bonten H, van Hooff H, Fiolet H, Buiting AG, Stobberingh EE. Infectious complications and antibiotic use in renal transplant recipients during a 1-year follow-up. *Clin Microbiol Infect.* 2001;7(11):619-625.

30. Kamath NS, John GT, Neelakantan N, Kirubakaran MG, Jacob CK. Acute graft pyelonephritis following renal transplantation. *Transpl Infect Dis.* 2006;8(3):140-147.

31. Abbott KC, Oliver JD, 3rd, Hypolite I, et al. Hospitalizations for bacterial septicemia after renal transplantation in the united states. *Am J Nephrol.* 2001;21(2):120-127.

32. Green H, Rahamimov R, Gafter U, Leibovitci L, Paul M. Antibiotic prophylaxis for urinary tract infections in renal transplant recipients: a systematic review and meta-analysis. *Transpl Infect Di.s* 2011;13(5):441-447.

33. Alangaden GJ, Thyagarajan R, Gruber SA, et al. Infectious complications after kidney transplantation: current epidemiology and associated risk factors. *Clin Transplant.* 2006;20(4):401-409.

34. Vidal E, Torre-Cisneros J, Blanes M, et al. Bacterial urinary tract infection after solid organ transplantation in the RESITRA cohort. *Transpl Infect Dis.* 2012;14(6):595-603.

35. Brennan DC. Cytomegalovirus in renal transplantation. *J Am Soc Nephrol.* 2001;12(4):848-855.

36. De Keyzer K, Van Laecke S, Peeters P, Vanholder R. Human cytomegalovirus and kidney transplantation: a clinician's update. *Am J Kidney Dis.* 2011;58(1):118-126.

Figure legend

Figure 1: Cumulative incidence of lower urinary tract infections in the first year after transplantation in both study groups

Figure 1

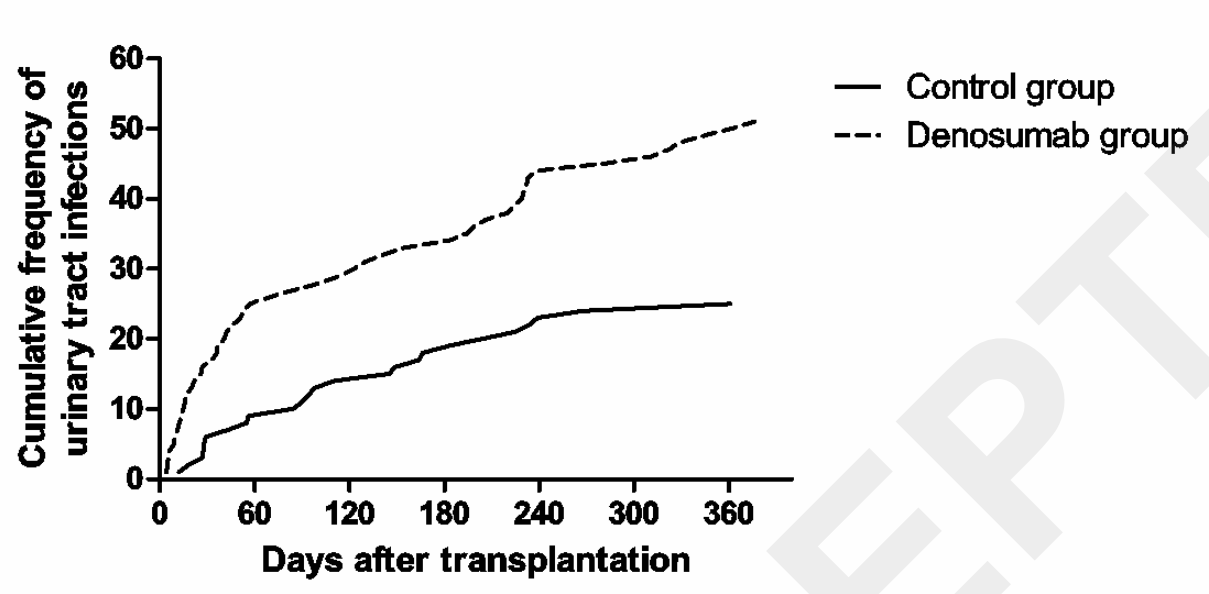

Table 1. Demographic and clinical characteristics of 90 study patients at baseline

Characteristic	**Control group (N=44)**	**Denosumab group (N=46)**
Age — years	49.0 (±12.9)	50.0 (±14.0)
Male sex — no. (%)	22 (50.0)	35 (76.1)
White ethnicity — no. (%)	42 (95.5)	44 (95.7)
Body mass index — kg/m^2	25.5 (±5.3)	25.8 (±4.6)
Pre-transplant dialysis mode — no. (%)		
Hemodialysis	31 (70.5)	26 (56.5)
Peritoneal dialysis	9 (20.5)	7 (15.2)
Pre-emptive transplantation	4 (9.1)	13 (28.3.)
Repeat transplantation — no. (%)	7 (15.9)	7 (15.2)
Transplant from deceased donor — no. (%)	26 (59.1)	18 (39.1)
No. of HLA mismatches	3.8 (±1.3)	3.5 (±1.3)
Panel reactive antibody titer ≥20% — no. (%)	0 (0.0)	0 (0.0)
Cause of end-stage renal disease — no. (%)		
Chronic glomerulonephritis	11 (25.0)	19 (41.3)
Diabetic nephropathy	3 (6.8)	4 (8.7)
Hypertensive/vascular nephropathy	6 (13.6)	2 (4.3)
Polycystic kidney disease	8 (18.2)	12 (26.1)
Other hereditary	4 (9.1)	1 (2.2)
Other	12 (27.3)	8 (17.4)
Immunosuppression — no. (%)		

Induction therapy †	44 (100.0)	45 (97.8)
Tacrolimus	33 (75.0)	30 (65.2)
Cyclosporine	11 (25.0)	16 (34.8)
Mycophenolate	44 (100.0)	46 (100.0)
Corticosteroids	44 (100.0)	46 (100.0)
CMV status		
High risk (D+/R-)	12 (27.3)	9 (19.6)
Intermediate risk (D+/R+, D-/R+)	28 (63.6)	24 (52.2)
Low risk (D-/R-)	4 (9.1)	13 (28.3)

Data are mean ± standard deviation or number (%). † Patients received basiliximab (68.9%) or anti-thymocyte globulin (30.0%).

Table 2. Infections in the study groups

Infections	**Control group (n=44)**		**Denosumab group (n=46)**	
	No. events (%)	**No. patients (%)**	**No. events (%)**	**No. patients (%)**
Urinary tract infection (cystitis)	25 (25.2)	11 (25.0)	51 (34.9)	24 (52.2)
Pyelonephritis/Urosepsis	5 (5.1)	5 (11.4)	3 (2.1)	3 (6.5)
Asymptomatic bacteriuria	0 (0)	0 (0)	1 (0.7)	1 (2.1)
CMV viremia[†]	24 (24.2)	18 (40.9)	26 (17.8)	20 (43.4)
Flu-like syndrome	14 (14.1)	13 (29.5)	17 (11.6)	12 (26.1)
BK viremia[††]	11 (11.1)	11 (25.0)	12 (8.2)	12 (26.1)
Herpes simplex type 1	4 (4.0)	4 (9.1)	8 (5.5)	5 (10.9)
Herpes zoster[†††]	0 (0)	0 (0)	3 (2.1)	3 (6.5)
Oral candidiasis	3 (3.0)	3 (6.8)	6 (4.1)	3 (6.5)
Mouth ulcer	3 (3.0)	3 (6.8)	3 (2.1)	2 (4.3)
Gastroenteritis	4 (4.0)	4 (9.0)	1 (0.7)	1 (2.1)
Wound infection	0 (0)	0 (0)	4 (2.7)	4 (8.7)
Cutaneous infection	0 (0)	0 (0)	4 (2.7)	4 (8.7)
Pneumonia	1 (1.0)	1 (2.3)	2 (1.4)	2 (4.3)
Sinusitis	1 (1.0)	1 (2.3)	2 (1.4)	2 (4.3)
Sigma diverticulitis	1 (1.0)	1 (2.3)	0 (0)	0 (0)
Parodontitis	1 (1.0)	1 (2.3)	0 (0)	0 (0)

Conjunctivitis	0 (0)	0 (0)	1 (0.7)	1 (2.1)
Retinitis	1 (1.0)	1 (2.3)	0 (0)	0 (0)
Otitis media	0 (0)	0 (0)	1 (0.7)	1 (2.1)
Gingivitis	1 (1.0)	1 (2.3)	0 (0)	0 (0)
Vaginal infection	0 (0)	0 (0)	1 (0.7)	1 (2.1)
Total	99 (100.0)	37 (84.1)	146 (100)	41 (89.1)

Data are number (%). † One case of CMV disease in the control group. †† One case of biopsy proven BK virus nephropathy in the denosumab group. ††† Classical herpes zoster along dermatomas.

Table 3. Type of urine bacteria in patients with urinary tract infections

Bacteria	**Control group**	**Denosumab group**	**Overall**
Escherichia coli	6 (24.0)	17 (33.3)	23 (30.3)
Klebsiella pneumoniae	6 (24.0)	6 (11.8)	12 (15.8)
Enterococcus faecalis	1 (4.0)	8 (15.7)	9 (11.8)
Coagulase-negative staphylococcus	0 (0)	4 (7.9)	4 (5.2)
Pseudomonas aeruginosa	2 (8.0)	1 (2.0)	3 (3.9)
Enterococcus faecium	1 (4.0)	0 (0)	1 (1.3)
Unknown†	2 (8.0)	1 (2.0)	3 (3.9)
Negative culture	0 (0.0)	9 (17.6)	9 (11.8)
Other	7 (28.0)	5 (9.8)	12 (23.5)
Total	25 (100.0)	51 (100.0)	76 (100.0)

Data are number (%). † No urine culture available but treated as urinary tract infection.

Table 4. Characteristics of CMV and BK virus infections

Patients with CMV viremia	**Control group**	**Denosumab group**	**p**
Patients with CMV viremia	18	20	p=0.805
High risk (D+/R-)	6	5	p=0.003
Intermediate risk (D+/R+, D-/R+)	12	14	
Low risk (D-/R-)	0	1	
CMV events			
Total number of events	24	26	
Primary infection	5	5	
CMV disease	1	0	
Time to CMV viremia occurrence after transplantation (days)	131.0 (41.5 – 260.0)	106.5 (43.3 – 149.5)	p=0.383
Maximum CMV copies/ml	7920 (2310 - 80477)	6170 (2492 - 35796)	p=0.668
Duration of positivity (days)	54 (35 - 170)	46 (28 - 69)	p=0.229
Treatment time (days)	100 (73 - 195)	109 (66 - 144)	p=0.699
Oral valganciclovir as primary therapy	11	23	
Intravenous ganciclovir as primary therapy	6	1	
No treatment due to a low virus replication (<2000 copies/ml) in intermediate risk constellation	7	2	

BKV viremia			
Patients with BK viremia	11	12	p=0.906
BK viremia (events)	11	12	
Induction with ATG	4	5	
Induction with basiliximab	7	6	
BK virus nephropathy	0	1	
Time to BK viremia occurrence after transplantation (days)	82 (53 - 83)	109 (73 - 156)	p=0.049
Maximum BK copies/ml	24172 (7046 - 299886)	28332 (1833 - 147218)	p=0.525
Duration of positivity (days)	183 (109 - 308)	172 (51 - 411)	p=0.833

Data are number, or median (interquartile range).